Policy Title :

Calibration of Centrifuges for Agglutination Enhancement

Department	Index No.	Scope
Laboratory & Blood Bank	**LAB-107**	**Blood Bank Staff**
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24/10/1432	**2**	**23/07/1440**
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01. Policy:

01.1. Centrifuges are used in serologic tests to enhance red blood cell antigen-antibody agglutination reactions. The amount of agglutination is dependent on the amount of centrifugation. Too little may produce a false weak or negative reaction and too much will pack the red cell button so tightly it is difficult to disperse. Centrifuges are calibrated to establish optimal centrifugation and avoid both extremes.

02. Definition:

02.1. N/A

03. Purpose :

02.1. Appropriate calibration of centrifuge gives accurate agglutination results..

04. Procedure :

04.1. **Material:**

04.1.1. Serologic centrifuges

04.1.2. Isotonic saline, 0.85% NaCl

04.1.3. Calibrated blood bank pipettes

04.1.4. 370C water bath

04.1.5. Glass test tubes

04.1.6. Volumetric pipettes and tips

04.2. **Reagent:**

04.2.1. In general, use serum containing an antibody that produces 1+ agglutination macroscopically. Prepare as fresh 3-5% suspensions of red blood cells. Select one sample of red cells positive for each antigen and one negative sample. (Below are recommended serum and cell suspensions. Slight modifications may be necessary so that a 1+ agglutination results).

04.2.2. Saline active antibodies.

04.2.3. 4ml serum from a group A person diluted with 25 to 30 parts 6% albumin (3ml 22% bovine albumin + 8ml normal saline).

04.2.4. Positive control – 3-5% Group B red cells in saline.

04.2.5. Negative control – 3-5% Group A red cells in saline.

04.2.6. Albumin active antibodies.

04.2.7. One-part anti-D diluted with 25-30 parts Rh-hr control (use 100ul anti-D + 2500 ul anti Rh-hr control).

04.2.8. Positive control – 2-5% D-positive red cells in saline.

04.2.9. Negative control – 2-5% D-negative red cells in saline

04.2.10. Antiglobulin Antibodies

04.2.11. Antiglobulin serum, unmodified

04.2.12. Positive control – prepare 3cc of a 2-5% saline suspension of D-positive cells. Add one drop of anti-D. Incubate at 370C for 15 minutes. Wash three times with saline. Resuspend cells in saline for a 2-5% suspension.

04.2.13. Negative control – use 3cc of the same 2-5% saline suspension of D-positive cells. Add one drop of 6% albumin. Incubate at 370C for 15 minutes. Wash three times with saline. Resuspend cells in saline for a 2-5% suspension.

04.3. **Method:**

04.3.1. Immediate spin and read reactions.

04.3.2. For each medium (saline, albumin and antiglobulin) prepare five tubes for positive reaction and a duplicate set for negative reactions.

04.3.3. Just before centrifugation, add two drops of the appropriate serum and one drop of the appropriate cell suspension to each labeled tube.

04.3.4. In pairs, one positive and one negative, centrifuge the tubes in a full loaded head for each of the following times: 10 seconds, 15 seconds, 20 seconds, 30 seconds, and 45 seconds.

04.3.5. Observe each tube for agglutination and record observations in a chart like the one below:

Criteria	Time in Seconds				
	1	1	2	3	4
Supernatant fluid clear	N	N	Y	Y	Y
Cell button clearly delineated	N	N	N	Y	Y
Cells easily resuspended	Y	Y	Y	Y	Y
Agglutination	±	±	1	1	1

Note:

- The optimum time of centrifugation is the least time required to fulfill these criteria.
- The supernatant fluid is clear.
- The red cell button is clearly delineated and the periphery is sharply defined, not fuzzy.
- The red cell button is easily resuspended.
- Agglutination is as strong as determined in preparing reagents, that is 1+. (In the example above, both 30 seconds and 45 seconds spins fulfill all the criteria, therefore, the optimum time for the centrifuge is 30 seconds).
- Washing procedure
- Fill all tubes used in the antiglobulin test section above with normal saline.
- In pairs, one positive and one negative, centrifuge tubes for each of the following times: 30 seconds, 45 seconds, 60 seconds, 90 seconds, and 120 seconds.
- All red blood cells should be in a clearly delineated button with no cells remaining up the side of the tube. The least time required to accomplish this is the optimum washing time for this centrifuge.

04.4. **Reporting of result:**

Report on "optimum spin time record" and file with other quality control reports

06. RESPONSIBILITY

06.1. All Blood Bank Staff of Al-Qunfudah General Hospital.

07. EQUIPMENT AND FORMS::

07.1. Maintenance of Refrigerator Centrifuge Form.

08. Attachment :

N/A

09. Reference

09.1. Clinical Lab Management for Clinical Lab Scientist

09.2. Technical Manual of the American Association of Blood Banks.

Preparation , Reviewing & Approval Box

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